Journal of Applied and Natural Science 7 (1) : 124 – 130 (2015)

JANS

Influence of egg parasitic fungus, *Engyodontium aranearum* against root knot nematode, *Meloidogyne incognita*

M. Muthulakshmi*, B. Anita and S. Subramanian

Department of Nematology, Tamil Nadu Agricultural University, Coimbatore – 641003, (Tamil Nadu) , INDIA
Corresponding author. E-mail: lakshminem@gmail.com

Received: November 14, 2014; Revised received: March 5, 2015; Accepted: March 15, 2015

Abstract: The indigenous egg parasitic fungal isolates, *Engyodontium aranearum* was evaluated for its nematicidal potential against root knot nematode, *Meloidogyne incognita*. The study revealed 53.75 per cent egg parasitization by the fungal isolate. Fungal colonies grew over the eggs and fungal hyphae penetrated the egg shells resulting in rupturing of egg shell layers, enzymatic digestion and empty eggs. The fungal culture filtrate was found to inhibit egg hatching by 83.42 per cent and caused upto 91.36 per cent juvenile mortality. This isolate also reduced the attraction of infective juveniles towards tomato root by 79.29 per cent. It seems to be a first report on the antinemic property of the fungus *E. aranearum* against root knot nematode, *M. incognita* and its effect was found comparable with *Paecilomyces lilacinus* which is known as an efficient nematode egg parasitic fungus.

Keywords: *Engyodontium aranearum*, Hatching, Mortality, *Paecilomyces lilacinus*, Parasitization, Root knot nematode

INTRODUCTION

Phytonematodes are being considered as serious limiting factor in the production of vegetables. Among the phytonematodes the root knot nematode, *Meloidogyne incognita* is reported to be responsible for 27.2 per cent yield loss in tomato (Jain *et al*., 2007). In the present scenario the biological control of pest including nematodes is gaining momentum due to ill effects of chemical nematicides and practical difficulties in adopting other methods of nematode management (Hague and Gowen, 1987). Recently an indigenous egg parasitic fungus isolated form potato in Ooty is was demonstrated as an potential bio candidate for the management of potato cyst nematode Muthulakshmi (2011).

The present study was programmed to study the influence and behavioural mechanism of the newly reported egg parasitic fungus on root knot nematode, *M. incognita.*

MATERIALS AND METHODS

Maintenance of monoculture of *M. incognita*: The seeds of tomato cv. Co 3 were surface sterilized by immersing in 0.1 per cent mercuric chloride for one minute and washed in distilled water for several times to remove the chemical. Five kg capacity pots were filled with autoclaved pot mixture (Red soil, sand and FYM at 1:1:1 ratio) and sterilized. Surface sterilized seeds were sown. After 25 days, the healthy seedlings were transplanted @ one seedling/ pot. Egg masses collected from *M. incognita* infested roots from the field were kept for hatching. The second stage juveniles were inoculated @ one J_2/gram of soil at 15 days after transplanting. The nematodes thus multiplied were maintained as pure culture. The nematodes required for the experimental purposes were harvested from this pure culture.

Maintenance of nematode egg parasitic fungi: The egg parasitic fungi, *E. aranearum* was maintained in the potato dextrose agar medium under room temperature (28 ±2°C) for 5-7 days. The strains were maintained on potato dextrose agar (PDA) and sub cultured on the same medium at 28°C in dark for two weeks. The fungus culture thus obtained was identified based on morphological characters and further used in laboratory and glasshouse experiments.

Mode of action: The mode of action of the *E. aranearum* isolates against root knot nematode, *M. incognita* was studied as follows:

Assessment of egg parasitization *in vitro*: Egg parasitism was measured using an *in vitro* bioassay, following the protocol described by Abrantes *et al.* (1998). The test was performed using *E. aranearum* against *M. incognita*. *Meloidogyne* eggs were obtained from egg masses cultured on tomato grown in a temperature-controlled glasshouse. The *M. incognita* eggs were separated. Briefly, egg parasitic fungal cultures growing on potato dextrose agar were flooded with 5 ml of sterile distilled water and aliquots of 0.2 ml of fungal suspension were spread on to Petri dishes (9 cm diameter) containing 0.8 per cent water agar with antibiotics after two days of incubation at room temperature. 100 root knot nematode eggs were added to each plate. The Petri dishes were incubated at room temperature (28±2°C) and after five days the numbers of parasitized eggs were counted under microscope.

Four plates were maintained for each treatment in completely randomized design (CRD)

In vitro* screening of *E. aranearum* culture filtrate against *M. incognita

Preparation of fungal culture filtrate: Culture filtrate of the egg parasitic fungal isolates was prepared in Erlenmeyer conical flask (250 ml) filled with 100 ml of potato dextrose (PD) broth medium and then sterilized by autoclaving at 15 lbs for 15 min. After sterilization, at lukewarm stage 50 mg of streptomycin sulphate was amended into the broth and mixed thoroughly. A fungal disc taken from the ten days old fungus was inoculated into the flasks containing medium and incubated at 28 ± 2°C for 15 days. After stipulated time the contents were filtered through Whatman No.1 filter paper and were subjected to centrifugation at 15000 g for 15 min. Centrifugation was done to remove the remaining hyphae and spores from the filtrates. The supernatant was designated as 100 per cent and from that required concentrations were prepared by adding distilled water for bioassay. The isolate of *Paecilomyces lilacinus* from Horticultural Research Station, Ooty was used as a biocontrol check in all the experiments. The effect of culture filtrates on egg hatching and juvenile mortality, attraction and penetration of RKN was studied *in vitro* in CRD.

Effect of *E. aranearum* on egg hatching ability of *M. incognita*: One ml of the fungal suspension of different concentrations (25, 50, 75 and 100 per cent) was transferred to 5.0 cm diameter Petri dishes and one egg mass of *M. incognita* was placed in each Petri dish and incubated at room temperature. Egg mass placed in distilled water and autoclaved plain broth served as untreated control and *P. lilacinus* as standard check. The numbers of hatched juveniles were counted after 24, 48 and 72 hrs of incubation. Four replications were maintained for each treatment in completely randomized design. The experiment was conducted under laboratory conditions.

Effect of *E. aranearum* on juveniles of *M. incognita*: One ml of the fungal suspension of different concentrations was transferred to 5.0 cm diameter Petri dishes. The *M. incognita* juveniles were transferred @ 100 J_2 in each Petri dish and incubated at room temperature (28±2°C). The juveniles placed in dishes containing sterile water and autoclaved plain broth served as control. The number of anesthetized nematodes were counted after 24, 48 and 72 hrs of exposure. The experiment was conducted in a CRD and each isolate was replicated four times.

Effect of *E. aranearum* on attraction of *M. incognita* in tomato: The attraction or repulsion effect of egg parasitic fungal isolates *M. incognita in vitro* was studied by agar plate method. Melted water agar (2%) was poured on the Petri dishes and kept in an incubator at a constant temperature of 27°C for 24hrs. Three circles of 3, 2 and 1 cm radius from the centre of Petri dish were drawn on the bottom of the Petri dishes denoting the regions of a, b and c respectively. The surface sterilized tomato seeds (cv. Co 3) were grown in sterilized sand medium. The roots of seedlings with 7 days old were dipped with *E. aranearum* suspension containing 10^8cfu/ml and placed in the centre of each of the Petri dishes with untreated root bits and without root bits to serve as control. The juveniles of *M. incognita* was inoculated in each Petri dish near the periphery @ 100 nematodes (J_2)/plate and kept inside the plant growth chamber at the temperature of 28 ± 2°C. The number of nematodes in the Petri plates was counted region wise at 24, 48 and 72 hrs after their introduction into the plates.

Effect of *E. aranearum* on *M. incognita* penetration: An experiment was conducted to study the influence of *E. aranearum* on root penetration of *M. incognita* under glasshouse conditions. Tumbler cups were filled with steam sterilized fine river sand. Surface sterilized tomato seeds (cv. Co 3) were treated with egg parasitic fungal isolates @ of 10 ml inoculum (containing 10^8cfu/ml) per kg seed. Then, the seeds were sown @ four seeds/ tumbler cup containing sterile sand. Untreated seeds served as control. The experiment was conducted in a completely randomized design and each treatment replicated four times. The cups were inoculated with 100J_2 of *M. incognita* one week after sowing. Plants from each cup were removed at an interval of one day starting from the day after inoculation (DAI) and continued upto 6 DAI. The roots were cut into small bits of 1 cm length, immersed in boiled lactophenol-acid fuchsin, destained in clear lactophenol and examined under microscope.

Statistical analysis: The data from various experiments were subjected to statistical analysis. The treatment means were compared by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984). The package used for analysis was IRRISTAT version 92-1 by International Rice Research Institute, Biometrics Unit, Philippines.

RESULTS AND DISCUSSION

Screening for parasitization of root knot nematode eggs by egg parasitic fungi: Two egg parasitic fungi were tested for their parasitic activity against root knot nematode eggs. Among the two fungi the highest egg parasitization was observed with *E. aranearum* (53.75 %) followed by *P. lilacinus* (51.35 %) compared to untreated control (Table 1). Fungal colonies grew over the eggs and the fungal hyphae penetrated the egg shells resulting in rupturing of egg shell layers, enzymatic digestion and empty eggs. Similar results were obtainted by Ayatollahy *et al*. (2008) where *P. chlamydosporia* var. *chlamydosporia* parasitized more than 70 per cent of the eggs in females and cysts on water agar. The fungus infected/colonized eggs were granular, dark brown and /or black in colour. Similar kind of egg parasitization was also reported by *P. chlamydosporia* in tomato (Sankarnarayanan *et al*., 2000) in *M. incognita* egg masses. Arora *et al.* (1990) observed the differences in the egg parastization ability among the 13 fungal isolates obtained from cysts in The Nilgiris. Highly significant differences

were noticed between isolates on the ability to parasitize nematode eggs *in vitro* and to colonize the rhizosphere of maize (Esteves *et al.,* 2009). In some cases the eggs became distorted even though the hyphae does not come in contact with the eggs, suggest a possible role of nematoxins. Nematophagous fungi may differ in their ability to penetrate the host cell by mechanical force and by producing various lytic enzymes (Stirling, 1991). Most of the colonized eggs were immature and therefore more susceptible to fungal invasion (Irving and Kerry, 1986). Mukhtar *et al.* (2013) revealed that the plant growth parameters and nematode infestations were recorded 7 weeks after inoculation. Both *P. penetrans* and *P. lilacinus* were equally effective and caused maximum reductions in number of galls, egg masses, nematode fecundity and build up as compared with *T. harzianum* and *P. chlamydosporia*. Our results indicate that application of antagonists can suppress galling and reproduction of *M.incognita* resulting in enhancement of plant growth.

In vitro* screening of *E. aranearum* against root knot nematode, *M. incognita

Inhibition in egg hatching: The two promising egg parasitic fungi were tested for their ovicidal effect against *M. incognita*. The experimental results revealed that the lowest egg hatching was observed in *E. aranearum* (40.14) followed by *P. lilacinus* (51.36) with 83.42 and 78.79 % inhibition in egg hatching respectively at cent per cent concentration of culture filtrate after 72 hrs exposure period (Table 2). Similar trend was observed upto 50 per cent concentration. The highest egg hatching was found in 25 per cent concentration at 72 hrs after exposure period with value of 69.64 and 65.91 per cent eggs hatched in *E. aranearum* and *P. lilacinus,* respectively compared with 50 per cent concentration. The highest egg hatching was recorded in control (distilled water) (242.13) followed by 25 per cent concentration of Potato dextrose broth (197.32) after 72 hr of exposure period. In the present study, the culture filtrate of the egg parasitic fungus *E. aranearum* significantly suppressed the hatching of root knot nematode eggs. Similar effects on hatching and juvenile mortality of cyst and root

Table 1. Parasitization of RKN eggs by egg parasitic fungal isolates.

S. N.	Treatments	Per cent eggs parasitized
1	*Engyodontium aranearum*	53.75^{a} (7.37)
2	*Paecilomyces lilacinus*	51.35^{b} (7.20)
3	Sterile water	0^{c} (0.71)
4	Control	0^{c} (0.71)
	SEd	0.0296
	CD (P=0.05)	0.0646

Values are mean of three replications, Figures in parentheses are √n+0.5 transformed value Column figures followed by different letters are significant different from each other at 5 per cent level by DMRT

Table 2. Effect of culture filtrate of egg parasitic fungus on *M. incognita* egg hatching

Treatments	Number of eggs hatched*											
	25%			50%			75%			100%		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
E. aranearum	45.36^{a} (63.20)	66.72^{a} (63.62)	73.51^{a} (69.64)	38.36^{a} (67.83)	56.33^{a} (69.29)	64.77^{a} (73.25)	29.75^{a} (75.05)	45.12^{a} (75.40)	52.56^{a} (78.29)	20.13^{a} (83.12)	37.63^{a} (79.48)	40.14^{a} (83.42)
P. lilacinus	51.98^{b} (57.83)	72.51^{b} (60.47)	82.54^{b} (65.91)	45.26^{b} (62.05)	63.71^{b} (65.27)	71.65^{b} (70.41)	36.12^{b} (69.71)	54.26^{b} (70.42)	67.10^{b} (72.29)	27.59^{b} (76.86)	48.03^{b} (73.81)	51.36^{b} (78.79)
Broth	110.46^{c} (10.38)	147.53^{c} (19.57)	197.32^{c} (18.51)	105.33^{c} (11.67)	134.12^{c} (26.88)	176.67^{c} (27.04)	95.12^{c} (19.40)	119.58^{c} (34.81)	166.54^{c} (31.22)	80.68^{c} (32.34)	97.73^{c} (46.72)	108.14^{c} (55.34)
Untreated control	119.25^{d}	183.42^{d}	242.13^{d}	119.25^{d}	183.42^{d}	242.13^{d}	119.25^{d}	183.42^{d}	242.13^{d}	119.25^{d}	183.42^{d}	242.13^{d}
S Ed	1.01	1.47	1.91	0.98	1.40	1.81	0.92	1.32	1.76	0.85	1.25	1.57
CD (P=0.05)	2.22	3.20	4.16	2.13	3.05	3.95	2.01	2.89	3.84	1.85	2.72	3.43

*Values are mean of four replications; Figures in parentheses are per cent decrease over control; In column means followed by a different letters are significantly different from each other t at 5 per cent level by DMRT

Table 3. Effect of culture filtrate of egg parasitic fungus on juveniles of *M. incognita.*

Treatments	Number of juveniles dead*											
	25%			50%			75%			100%		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
Engyodontium	28.47^{a}	42.48^{a}	58.87^{a}	46.54^{a}	60.12^{a}	68.76^{a}	65.36^{a}	76.52^{a}	82.57^{a}	75.45^{a}	83.67^{a}	91.36^{a}
aranearum	(5.38)	(6.56)	(7.71)	(6.86)	(7.79)	(8.32)	(8.12)	(8.78)	(9.11)	(8.71)	(9.17)	(9.58)
Paecilomyces	23.52^{b}	37.37^{b}	52.12^{b}	37.05^{b}	52.73^{b}	60.37^{b}	61.62^{b}	68.37^{b}	75.28^{b}	71.05^{b}	78.15^{b}	86.56^{b}
lilacinus	(4.90)	(6.15)	(7.25)	(6.13)	(7.30)	(7.80)	(7.88))	(8.30)	(8.70)	(8.46)	(8.87)	(9.33)
Broth	0^{c}	0^{c}	0.67^{c}	1^{c}	1.46^{c}	2.01^{c}	2.96^{c}	3.87^{c}	4.52^{c}	4.81^{c}	5.34^{c}	6.92^{c}
	(0.71)	(0.71)	(1.08)	(1.22)	(1.40)	(1.58)	(1.86)	(2.09)	(2.24)	(2.30)	(2.24)	(6.92)
Untreated	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}
control	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)
S Ed	0.02	0.02	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
CD (P=0.05)	0.04	0.05	0.06	0.05	0.06	0.07	0.07	0.07	0.07	0.07	0.08	0.08

*Values are mean of four replications, Figures in parentheses are √n+0.5 transformed values;In column means followed by a different letters are significantly different from each other at 5 per cent level by DMRT

knot nematodes due to egg parasitic fungi was reported by Shinya *et al*. (2008) and Regaieg *et al*. (2010).A number of nematophagous fungi are known to have proteolytic and chitinolytic activities which cause alteration in eggs cuticular structure, changes in egg shell permeability or cause perforations in the cuticle which allows seepage of toxic metabolites into the eggs and cause physiological disorders (Webb *et al*., 1972; Jatala *et al*., 1985; Lopez -Llorca, 1990). These factors may have important role in the inhibition of egg hatching of root knot nematode. Consequently, the eggs (*G. pallida*) lose permeability and strength, becoming deformed and swollen (Tikhonov *et al.,* 2002). During its primary infection steps, this fungus produces an alkaline serine protease that specifically degrades the proteinaceous outer-vitelline membrane of the eggs (Morton *et al*., 2004). Similarly, toxin-producing fungi affect nematodes by the production of nematicidal compounds (Dong *et al*., 2006). A scanning electron microscopy study of treated eggs showed severe alterations caused by the filtrate of isolate HR43 (*P. chlamydosporia*) on *M. incognita* eggs, which appeared collapsed and not viable, suggesting the production of chitin-degrading enzymes or other active compounds (Regaieg *et al*., 2010).

Influence on juveniles of *M. incognita:* The egg parasitic fungi were tested at 25, 50, 75 and 100 per cent concentration at different exposure periods of 24, 48 and 72 hrs interval against *M. incognita* juveniles. The results revealed that there was a gradual increase in the mortality of *M. incognita* juveniles with an increase in the concentration of different culture filtrates of egg parasitic fungi and its period of exposure compared to control (Distilled water) . The isolate *E. aranearum* at 100 per cent concentration caused mortality (91.36 juveniles) at 72 hrs exposure period and it was followed by *P. lilacinus* (86.56 juveniles) (Table 3). The *E. aranearum* (76.52) and *P. lilacinus* (68.37) at 75 per cent concentration were found to cause juvenile mortality at 48 hrs exposure period. The least juvenile mortality was observed with *E. aranearum* (28.47) followed by *P. lilacinus* (23.52) at 25 per cent concentration after 24 hrs exposure. The results revealed there was a positive correlation exists between per cent mortality of juveniles of *M. incognita* and *E. aranearum* concentration/time of exposure. The highest egg hatching was recorded in control (distilled water) (242.13) followed by 25 per cent concentration of potato dextrose broth (197.32) after 72 hrs of exposure period. In the present study, the culture filtrate of the egg parasitic fungus *E. aranearum* significantly caused high juvenile mortality. Reddi Kumar *et al.* (2008a), Shinya *et al*. (2008) and Regaieg *et al*. (2010) revealed that lower number of root knot nematode and cyst nematode eggs hatched and higher juvenile mortality was observed using egg parasitic fungi.

Effect of culture filtrate of *E. aranearum* on attraction of *M. incognita* juveniles: The isolate *E. aranearum* reduced juveniles attraction towards tomato root by 79.29 per cent over control and it was followed by *P.*

Table 4. Effect of cent per cent culture filtrate of egg parasitic fungus on attraction of *M. incognita* juveniles in tomato.

Treatments	Number of J_2 attracted*					
	24 hrs	Percent decrease over control	48hrs	Percent decrease over control	72 hrs	Percent decrease over control
Engyodontium aranearum	12.15[a]	79.29	27.56[a]	67.71	38.05[a]	59.33
Paecilomyces lilacinus	17.84[b]	69.59	33.97[b]	60.20	44.17[b]	52.79
Broth	55.76[c]	4.96	80.27[c]	5.96	88.49[c]	5.43
Untreated control	58.67[d]	-	85.36[d]	-	93.57[d]	-
S Ed	0.48	-	0.72	-	0.81	-
CD (P=0.05)	1.05	-	1.57	-	1.77	-

*****Values are mean of four replications; In column means followed by a different letters are significantly different from each other at 5 per cent level by DMRT.

lilacinus (69.59 %) at 24 hrs of exposure period. The highest attraction was observed in control (distilled water) (93.57) followed by broth (88.49) after 72 hrs exposure period (Table 4). The results revealed that the highest percentage of nematode attraction was found in 24 hrs exposure period when compared to all other periods of exposure *viz*., 48 and 72 hrs. The nematode attraction towards host root decreased in all the treatments with increase in the exposure time. The results of the *in vitro* studies indicated that *E. aranearum* treated tomato seedlings reduced *M. incognita* attraction towards tomato roots compared to untreated control. The mechanisms of action may be due to the reduction of attraction and or repellence by production of metabolites and/or degradation of specific root exudates which control nematode behavior. Similar results were obtained by Dababat (2007) and Dababat and Sikoro (2007) who reported that the endophytes inoculated plants exudates reduced the total number of nematodes attracted to the inoculation area by upto 80 per cent over uninoculated control. The results indicated that the application of *P. chlamydosporia* enriched vermicompost at the rate of 50g/ m^2 was significantly effective in reducing the population of *Meloidogyne incognita* and increase the yield by 23.08 per cent on okra. (Chaya and Rao, 2012).

Effect of *E. aranearum* culture filtrate on *M. incognita* juveniles penetration in tomato root: The nematode penetration in tomato roots was observed upto 6 days after inoculation (DAI). The results revealed that level of nematode penetration was reduced by 87.50, 85.57, 83.10, 77.02, 65.43 and 60.74 per cent in *E. araneraum* treated seedlings over control at 1, 2, 3, 4, 5 and 6 DAI respectively. It was followed by *P. lilacinus* (82.81, 80.60, 70.06, 66.77, 56.56 and 51.85) and differed in significantly (P<0.05) over control (Table 5). The results revealed that *E. aranearum* and *P. lilacinus* reduce had profound effect to the root penetration of *M. incognita* to tomato. The highest nematode penetration was observed in control (91.54) followed by broth (88.76). The results of the *in vitro* studies indicated that significant (P<0.05) reduction in penetration of *M. incognita* in *E. aranearum* treated tomato plants. Earlier, Oostendrop and Sikora (1990) reported that the sugar beet cyst nematode, *Heterodera schachtii* penetration was decreased due to *P. fluorescens* treatment. The mechanism responsible for the reduction in nematode penetration was attributed to the ability of the bacterium to envelop or bind to root surface lectins, thereby interfering with normal host recognition by the nematode as also reported by Siddiqui and Mahmood (1995) and Kalaiarasan (2000) for root knot nematode.

Conclusion

The present study concludes that the egg parasitic fungus, *E. aranearum* is a potential biocontrol agent against root knot nematode, *M. incognita* . This fungus was found to parasitize nematode eggs leading to reduction in hatching and juvenile mortality. The studies also indicated that *E. aranearum* treated tomato roots attracted less *M. incognita* juveniles. The *E. aranearum* against root knot nematode was reported for the first time in India.

ACKNOWLEDGEMENTS

The authors are thankful to Societal Research Fellowship Scheme (WOS-B), Department of Science and Technology, who provided necessary funds operating the scheme work.

REFERENCES

Abrantes, I., Alho, L, Bourne, J., Carella, A., Ciantio, A., Clara, M.I.E., Davies, K., Franco, C., Hirch, P., Kerry, B., Lamberti, F., Lopez-Llorca, L., Mota, M., Santos, C., Sasanelli, N., Sorribas, J., Tzortzakakis, E.A. and Verdejo-Lucas, S. (1998). A workshop manual for research on *Verticillium chlamydosporium* as a biological control agent for root knot nematodes. IACR, Rothamsted, U.K.

Arora, R.K., Singh, D.K. and Joseph, M.M. (1990). Parasitization of potato cyst nematode eggs by fungi associated with cysts. *Indian Phytopathology,* 43: 294-295.

Ayatollahy, E., Fatemy, S. and Etebarian, H.R. (2008). Potential for biocontrol of *Heterodera schachtii* by *Pochonia chlamydosporia* var. *chamydosporia* on sugar beet. *Biocontrol Science and Technology,* 18: 157-167.

Chaya, M.K. and Rao, M.S. (2012). Bio-management of *Meloidogyne incognita* on okra using a formulation of

Table 5. Effect of cent per cent culture filtrate of egg parasitic fungus on root penetration by *M. incognita* juveniles in

Treatments	Number of J_2 penetrated*											
	1 DAI	Per cent decrease over control	2 DAI	Per cent decrease over control	3 DAI	Per cent decrease over control	4 DAI	Per cent decrease over control	5 DAI	Per cent decrease over control	6 DAI	Per cent decrease over control
E. aranearum	7.36^{a}	87.50	9.42^{a}	85.57	12.28^{a}	83.10	18.63^{a}	77.02	30.67^{a}	65.43	35.94^{a}	60.74
P. lilacinus	10.12^{b}	82.81	12.67^{b}	80.60	21.76^{b}	70.06	26.94^{b}	66.77	38.54^{b}	56.56	44.08^{b}	51.85
Broth	50.35^{c}	14.47	57.26^{c}	12.34	65.72^{c}	9.56	76.94^{c}	5.09	81.03^{c}	8.68	88.76^{c}	3.04
Untreated control	58.87^{d}	-	65.32^{d}	-	72.67^{d}	-	81.07^{d}	-	88.73^{d}	-	91.54^{d}	-
S Ed	0.45	-	0.51	-	0.58	-	0.67	-	0.74	-	0.80	-
CD (P=0.05)	0.98	-	1.11	-	1.26	-	1.46	-	1.63	-	1.75	-

*Values are mean of four replications, Figures in parentheses are % decreased over control; In column means followed by a different letters are significantly different from each other at 5 per cent level by DMRT.

Pochonia chlamydosporia. *Pest Management in Horticultural Ecosystems, 18(1):* 84-877.

Dababat, A.A. and Sikora, R.A. (2007). Effects of the mutualistic endophyte *Fusarium oxysporum* 162 on *Meloidogyne incognita* attraction and penetration on tomato. *Nematology,* 9: 771-776.

Dababat, A.A. (2007). Importance of the mutualistic endophyte *Fusarium oxysporum* 162 for enhancement of tomato transplants and the biological control of the root knot nematode *Meloidogyne incognita*, with particular reference to mode-of-action. Dissertation, Rheinische Friedrich -Wilhelms-Universitat, Bonn, Germany.

Dong, J.Y., Zhou, Y., Zhou, L.R., Zhu, L.L., Huang, R. and Zhang, K.Q. (2006). New nematicidal azaphilones from the aquatic fungus *Pseudohalonectria adversaria* YMF1.01019. *FEMS Microbiology Letters,* 264: 65-69.

Esteves, I., Peteira, B., Atkins, D.S., Magan, N. and Kerry. B. (2009). Production of extracellular enzymes by different isolates of *Pochonia chlamydosporia*. *Mycological Research,* 113: 867-876.

Gomez, K.A. and Gomez. A.A. (1984). Statistical Procedure for Agricultural Reasearch John Wiley and Sons, New York.

Hague, N.G.M. and Gowen. S.R. (1987). Chemical control of nematodes. *In: Principles and Practice of Nematode Control in Crops*. Brown, R.H., Kerry, B.R. (Eds.). Australia Academic Press, pp. 133-178.

Irving, F. and Kerry. B.R. (1986). Variation between strains of the nematophagous fungus *Verticillium chlamydosporium* Goddard II. Factors affecting parasitism of cyst nematode eggs. *Nematologica,* 32: 474-485.

Jain, R.K., Mathur, K.N. and Singh. R.V. (2007). Estimation of losses due to plant parasitic nematodes on different crops in India. *Indian J. Nematol.,* 37: 219-221.

Jatala, P., Franco, J., Gonzales, A. and O'Hara. C.M. (1985). Hatching stimulation and inhibition of *Globodera pallida* eggs by enzymatic and exopathic toxic compounds of some biocontrol fungi. *Journal of Nematology,* 17: 501.

Kalaiarasan, P. (2000). Studies on the nematode pest of groundnut (*Arachis hypogaea* L.) and its management. M.Sc., (Ag.,) Thesis, TNAU, Coimbatore-3, India. pp 93.

Lopez-Llorca, L.V. (1990). Purification and properties of extracellular proteases produced by the nematophagous fungus, *Verticillium suchlasporium*. *Canadian Journal of Microbiology,* 36: 530-537.

Morton, O.C., Hirsch, P.R. and Kerry. B.R. (2004). Infection of plant parasitic nematodes by nematophagous fungi - a review of the application of molecular biology to understand infection processes and to improve biological control. *Nematology,* 6: 161-170.

Mukhtar, T., Arshad Hussain, M. and Zameer Kayani, M. (2013). Biocontrol potential of *Pasteuria penetrans*, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Meloidogyne incognita* in okra. *Phytopathologic Mediterranea,* 52(1): 66-76.

Oostendorp, M. and Sikora. R.A. (1990). *In vitro* interrelationship between rhizosphere bacteria and *Heterodera schachtii*. *Rev. Nematol.,* 13: 269-274.

Reddi Kumar, M., Sailaja Rani, J. and John Sudheer. M. (2008). Effect of culture filtrates of *Paecilomyces lilacinus* on the mortality and hatching of root knot nematode, *Meloidogyne incognita*. *Indian Journal of Nematology,* 38: 90-93.

Regaieg, H., Ciancio, A. Raouani, N.H. Grasso, G. and Rosso. L. (2010). Effects of culture filtrates from the nematophagous fungus, *Verticillium leptobactrum* on viability of the root knot nematode, *Meloidogyne incongita*. *World Journal of Microbiology and Biotechnology,* 26; 2285-2289

Sankaranarayanan, C., Hussaini, S.S., Kumar, P.S. and Rangeshwaran. R. 2000. Biological control of *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood 1949 on tomato by *Verticillium chlamydosporium* Goddard cultured on different substrates. *Journal of Biological Control,* 14: 39-43.

Shinya, R., Aiuchi, D., Kushida, A., Tani, M., Kuramochi, K. and Koike. M. (2008). Effects of fungal culture filtrates of *Verticillium lecanii* (*Lecanicillium* spp.) hybrid strains on *Heterodera glycines* eggs and juveniles. *Journal of Invertebrate Pathology,* 97: 291-297.

Siddiqui, Z.A. and Mahmood. I. (1995). Management of *Meloidogyne incognita* race 3 and *Macrophomina phaseolina* by fungus culture filtrates and *Bacillus subtilis* on chickpea. Fundam. *Appl. Nematol.,* 18: 71-76.

Stirling, G.R. (1991). Biological control of plant parasitic nematodes- Progress, problems and prospects. CAB International, Wallingford, UK. pp 282.

Tikhonov, V.E., Lopez-Llorca, L.V. Salinas, J. and Jansson. HB. (2002). Purification and characterization of chitinases from the nematophagous fungi, *Verticillium chlamydosporium* and V. *suchlasporium*. *Fungal Genetics and Biology,* 35: 67-78.

Webb, H.M., Ghafoor, A. and Heale, J.B. (1972). Protein and enzyme patterns in strains of *Verticillium. Transactions of the British Mycological Society,* 59: 393-402.